

**Note to Readers:** *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact [ehp508@niehs.nih.gov](mailto:ehp508@niehs.nih.gov). Our staff will work with you to assess and meet your accessibility needs within 3 working days.

## **Supplemental Material**

### **Effects of Common Pesticides on Prostaglandin D2 (PGD2) Inhibition in SC5 Mouse Sertoli Cells, Evidence of Binding at the COX2 Active Site, and Implications for Endocrine Disruption**

Subramaniam Kugathas, Karine Audouze, Sibylle Ermler, Frances Orton, Erika Rosivatz, Martin Scholze, and Andreas Kortenkamp

#### **Table of Contents**

**Figure S1:** Chemical structures of tested pesticides

Pesticides inhibiting PGD2 synthesis in SC5 cells

Pesticides tested but not inhibiting PGD2 synthesis in SC5 cells

Common NSAIDs

**Figure S2:** Suppression of PGD2 synthesis by pesticides in the mouse Sertoli cell assay. The graphs show data, best-fitting models (solid line) and 95% confidence belts (dashed line) for PGD2 synthesis inhibiting responses in SC5 (mouse Sertoli) cells after 24 hours exposure (3 replicates). All data were normalised to those of solvent controls.

**Figure S3:** Lack of cytotoxicity of test agents in the concentration ranges associated with PGD2 suppression. Shown are MTT responses (absorbance at 570 nm) in SC5 cells normalised to solvent-treated controls (absorbance readings in controls were set to 100%). The range associated with PGD2 suppression is depicted by the grey boxes. A clear downward trend in responses was not seen over the concentration ranges investigated.

**Figure S4:** Time course of PGD2 suppression in SC5 cells by OPP, ibuprofen and aspirin. SC5 cells were exposed to ibuprofen (IBU), aspirin (ASP) and o-phenylphenol (OPP) at their respective IC<sub>50</sub> concentrations (128 nM, 3426 nM and 175 nM of ibuprofen, aspirin and OPP, respectively) for the indicated time periods, and PGD2 concentrations measured. For each time point, PGD2 levels (average of 3 experiments performed in duplicate) are expressed as percentage of solvent controls. The last time point is 24 hours.

**Figure S5:** Effect of AA supplementation on the inhibition of PGD2 synthesis by ibuprofen and o-phenylphenol. PGD2 synthesis in mouse Sertoli cells after 24 h exposure to ibuprofen (black solid line) and o-phenylphenol (black dashed line) without AA. Supplementation of AA (10 µM) during the last 2 hours of the 24 h exposure duration, with ibuprofen (blue solid line) or o-phenylphenol (blue dashed line). The inset figure (red line) shows the dose response curve of AA stimulation of PGD2 synthesis. The graphs show mean responses (dots, n=3) and their best-fitting regression models. Data were normalised against solvent controls.

**Table S1:** Different endocrine disrupting compounds and their IC<sub>50</sub> values in PGD2 inhibition and AR-antagonism assays. Values for ethylparaben, *n*-propylparaben, *n*-butylparaben, benzophenone 3, bisphenol A, flutamide and *p,p'*-DDE (PGD2 inhibition) were obtained from Kristensen et al. (2011 b), and AR antagonism from Ermler et al. (2011). The AR antagonism data for all remaining chemicals are from Orton et al. (2011), those for PGD2 inhibition are data reported here (shown in bold).

## References